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Histological Observations on Some Visceral Organs of an Oil-polluted Ancient Murrelet *Synthliboramphus antiquus* (Aves) stranded on The Niigata Coast

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Abstract

Anatomical examination was carried out on some organs of a fresh dead body of the oil-polluted ancient murrelet, *Synthliboramphus antiquus,* stranded on the Niigata Coast at the Sea of Japan in mid winter. Of the alimentary organs examined, the proventriculus showed a severe derangement of the mucous membrane and the proventricular gland, probably due to toxic effects of pollutants ingested through the act of preening. The epithelial layers of the proventriculus were affected extensively and characterized by the presence of the fallen necrotic cells and their debris. A sign of necrosis was also detected in some of the esophageal glands. However, no anomalous changes were noted in other parts of the alimentary canal, exocrine pancreas, and liver. The adrenal cortex showed a hyperfunctional state as evidenced by hypertrophy of the cortical cells with occasional mitotic figures. The hypophysis and the thyroid appeared to be in a normal healthy condition. Thus, the present study demonstrated the toxic effects of external oiling on some visceral organs of the ancient murrelet.

Introduction

The serious hazard of oil pollution to seabirds in estuarial and coastal waters has been well documented (Bourne, 1968 ; Clark, 1968 ; Nelson-Smith, 1970). Two major factors appear to be involved in this hazard : the external oiling of the birds and the ingestion of the oil through the act of preening. The former has direct effects on the plumage, i.e., losses of waterproofing, insulation, and buoyancy (Hartung, 1967 ; McEwan and Koelink, 1973) ; whereas the latter leads to various pathological conditions : fatty degeneration of the liver, toxic nephrosis, enlargement of the spleen, adrenocortical hyperplasia, acinar atrophy of the pancreas, symptoms of intestinal irritation, and lipid pneumonia (Hartung and Hunt, 1966 ; Guillon, 1967). Practical methods for rescue and conservation (cleaning and rehabilitation) of the polluted birds have also been designed and attempted (Clark, 1968 ; 1978).

In contrast to a growing body of information about oiled seabirds in the northern waters of the Atlantic and Pacific, very littlle is known about the oiled seabirds in the Japanese waters irrespective of actual incidences of oil spillage (Kikuchi, 1972 ; Honma and Kitami, 1974 ; Aoki *et al*., 1975). Previous papers (Kazama, 1971 ; Chiba, 1974) reported strandings of oiled and non-oiled seabirds on the Niigata Coast of the Sea of Japan during winter, in particular after a stormy monsoon. However, the physical condition of such polluted seabirds has remained unexplored. The aim of the present study was to provide information on the macroscopic and microscopic anatomy of some visceral organs of a fresh dead ancient murrelet, *Synthliboramphus antiquus*, the death of which was probably by oil pollution.

Materials and methods

A fresh dead body of the ancient murrelet was selected for histological study from more than 10 dead murrelets stranded on the sandy beach of Niigata Coast at the Sea of Japan in mid January, 1973 (Chiba, 1974). Most of the birds including the present specimen were polluted variably by petroleum, probably waste oil. The measurements of the specimen were as follows : body weight (g), 168 : wing (mm)142.0, tail 46.0, tarsus

26.0, bill 13.0. Visceral organs, mainly alimentary and endocrine organs, were quickly dissected out and immersed in Bouin's fluid for a few days. The organs and tissues were appropriately trimmed, dehydrated through a graded series of ethanol, embedded in paraffin, and cut serially at an 8-μm thickness. The sections were routinely stained with Mayer's hematoxylin-eosin, azan trichrome, or periodic acid Schiff's reagent (PAS) and counterstained with fast green (FG)-orange G (OG) in the case of PAS or with aldehyde fuchsin (AF)-FG-OG stain, and examined under a light microscope.

Results

Macroscopic anatomy

Nearly the caudal entire half of the abdomen was moderately covered with petroleum. The weight (168g) of the bird was light, there being no notable fat deposition under the skin. A functional left ovary in undeveloped condition was seen as a compact flattened body, lying close to the cephalic portion of the kindney. The stomach appeared as a slender structure as if it was empty, but it contained a small amount of dark chyme-like material in the cavities of both glandular and muscular portions of it (Fig.1). The color of the stomach contents was closely similar to that of the pollutant on the abdominal feathers, strongly suggesting that the material included components of petroleum taken through the preening. There was almost no fat deposition in the viscera, but no anomaly was seen in the visceral organs at the macroscopic level.

Microscopic anatomy

Esophagus: No severe derangement was seen in the esophagus. The cells of the stratified squamosus epithelium were compactly arranged and contained acidophilic cytoplasm and an ovoid nucleus with a prominent nucleolus (Fig.2). The mucous glands were flask-like in shape, distributed widely and beneath the mucosa, and had a spacious cavity, in which a fluid-like material was occasionally seen. The columnar gland cells had rich cytoplasm with an alveolar feature. A small densely stained nucleus was located in the basal region of the cell. In some glands, the mucous cells were highly vacuolated and distorted, suggesting a necrotic change. No anomalous chang was seen in the submucosa or in the muscular tunic.

Stomach: Necrotic changes were evident in the epithelial layers of the proventriculus (the glandular part). The mucous membrane and the proventricular glands were markedly distorted and injured (Fig. 3). The epithelial components were largely detached from the connective tissue and had fallen into the cavity. The detached mucous cells still showed a positive PAS reaction for polysaccharides, but deterioration of the mucous cells, duct cells, and zymogenic cell, and also of the laminar propria, was evident under high-power view (Fig. 4). On the other hand, only small parts of the epithelium lying in the deeper region appeared to be histologically normal. Here, most of the zymogenic cells were thin and contained fewer granules, but no severe derangement was seen in the submucosa and muscular layers. In addition, microscopic examination of the dark chyme-like material in the stomach cavity showed that the material comprised unidentifiable debris (Fig. 5). Minute clear droplets, amorphous material, and dark metal-like substances were barely discerned. The gizzard (the muscular part) was not examined histologically.

Intestine: No noteworthy changes were seen throughout subdivisions of the intestine (Fig. 6), although a small amount of the dark chyme-like material was sporadically encountered in the cavity and on the mucous membrane. The following parts of the intestine were diagnosed to be normal: the columnar epithelium containing numbers of goblet cells, glands, gut-associated lymphoid tissue, submucosal connective tissue, circular and longitudinal muscle layers, and serous investment. It was interesting to note clumps of dark granules or droplets in the apical parts of the absorptive epithelium (Fig. 7). These structures were tinted brown or dark brown in unstained sections.

Liver: The liver, a bilobed volminous organ, weighed 5.9g, constituting 3.5% of the body weight. No histological anomalies were noted in the hepatic tissue. The polygonal parenchymatous cells contained rich cytoplasm and a vesicular nucleus with a prominent nucleolus (Fig. 8), but some cells were vacuolated or had been shrunken. In the majority of the cell, the cytoplasm was coarsely granulated and stained intensely with acidic dye. Apart from the parenchymatous cells, densely granulated cells were sporadically encountered in close association with the capillaries or sinusoids. These cells were tentatively diagnosed as granular leucocytes and/or macrophages.

Exocrine pancreas: The exocrine pancreas also showed a normal picture (Fig. 9).

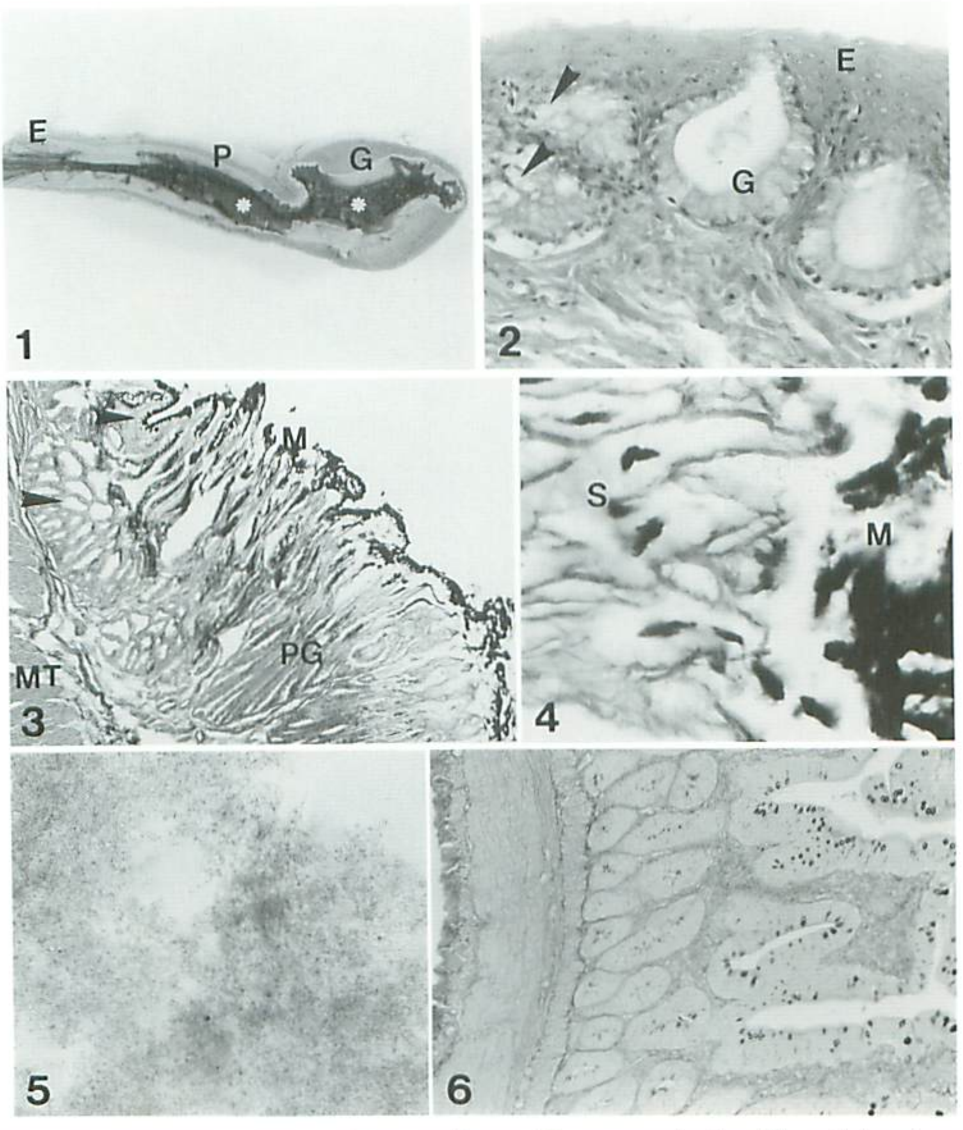

Fig.1. Macroscopic features of the esophagus (E), proventriculus (P), and the gizzard (G) of an oil-polluted ancient murrelet, showing the dark chyme-like substance (*) in the stomach cavity. ×0.8

Fig.2. A part of the esophagus, showing normal histology of the epithelium (E) and some of the mucous glands (G). Note a sign of necrosis (arrowheads) in some glands. Hematoxylin-eosin stain. ×300

Fig.3. A part of the proventriculus showing a severe derangement of the mucous membrane (M) and the proventricular glands (PG). Note that a small part (arrowheads) remained unaffected by the ingested pollutant. MT, muscular tunic. PAS-FG-OG stain. ×120

Fig.4. High-power view of the necrotic changes of the proventricular epithelium, which included clusters of detached mucous cells (M) and a markedly distorted submucosa (S). PAS-FG-OG stain. ×700

Fig.5. Smear preparation of the ingested pollutant remaining in the stomach cavity. × 150

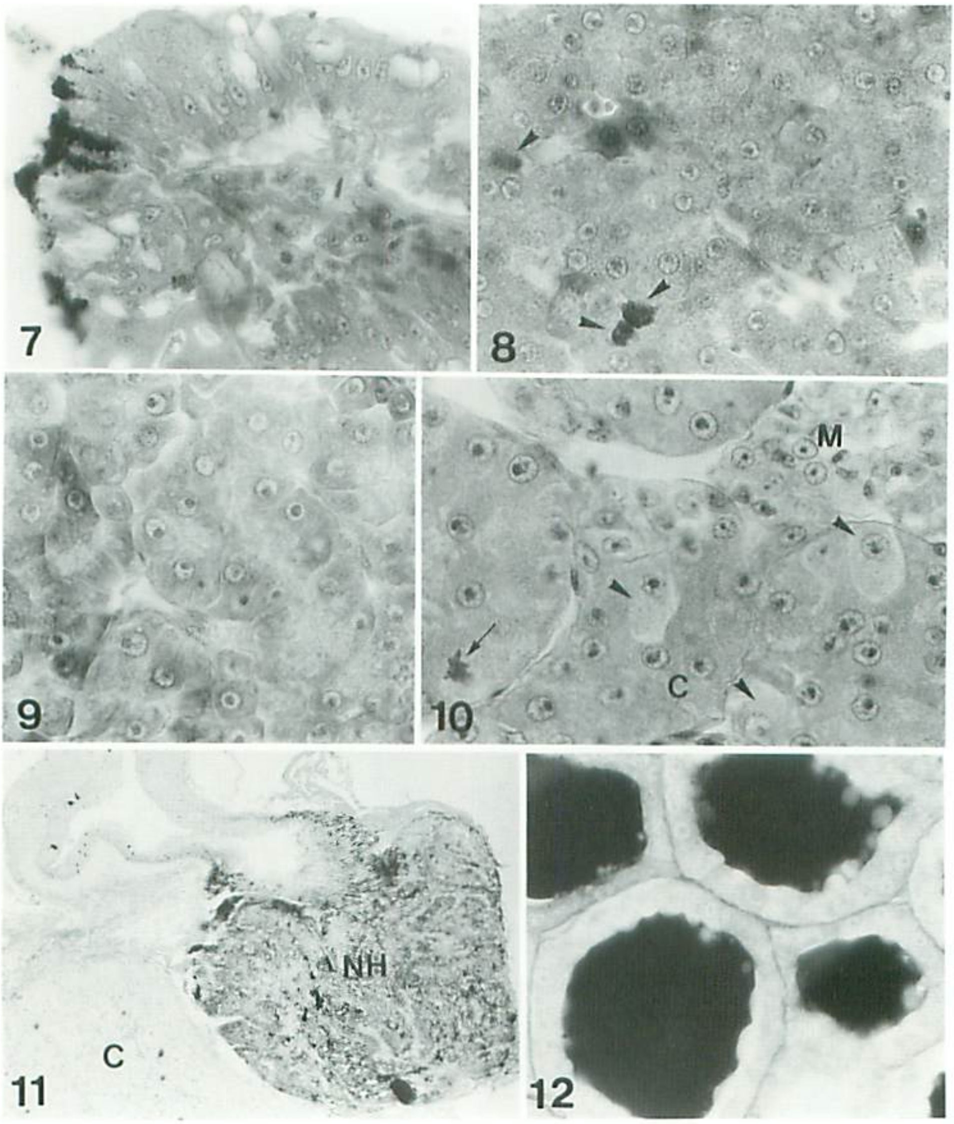

Fig.6. A part of the intestine (the ileum), showing normal histological features. PAS-FG-OG stain. ×200

Fig.7. A part of the intestinal villi, showing dark material attatched to the absorptive epithelium. Hematoxylin-eosin stain. ×700

Fig.8. A part of the liver, showing the healthy parenchymatous cells and scattered granulated cells (arrowheads). Hematoxylin-eosin stain. ×700

Fig.9. A part of the exocrine pancreas, showing healthy acinar cells. Heamtoxylin-eosin stain. ×700

Fig.10. A part of the adrenal gland, showing hyperplastic cortical tissue (C). Note the hypertrophied cortical cells (arrowheads) and a mitotic figure (arrow). M, medullary tissue. Hematoxylin-eosin stain. ×700

Fig.11. A part of the hypophysis, showing the neurohypophysis (NH) containing a moderate amount of neurosecretory (AF-positive) material. C, caudal lobe of the pars distalis. AF-FG-OG stain. ×50

Fig.12. A part of the thyroid gland, showing a healthy epithelium and plenty of colloid in the follicular lumen. AF-FG-OG stain. ×700

The acinar cells were moderately or intensely stained with hematoxylin and contained numbers of eosinophilic coarse granules, indicating that the cells are rich in basophilic substance (ribosomes) and zymogen granules. The nucleus was round and the nucleolus was very prominet. No detailed examination was made on the endocrine pancreas, although AF-positive B (insulin) cells were easily indentified.

Adrenal gland : The components of the adrenal gland, i.e., the cortex and medulla, were well demarcated, although they were intermingled with each other. The cortex appeared to be in a hyperfunctional state ; the majority of the cortical cells were hypertrophied, contained a plenty of acidophilic cytoplasm, and showed a spherical nucleus with a distinct nucleolus. In addition, mitotic figures were occasionally found (Fig. 10).

Other endocrine glands : No anomalous or pathological changes were observed in the hypophysis and thyroid gland. The neurohypophysis was moderately or less intensely stained with AF, implying the presence of a moderate amount of neurohypophyseal hormone and neurophysin in the neurohaemal sites (Fig. 11). The thyroid gland appeared to be healthy and was concluded to have been a moderate state of secretory activity ; the follicle cells were cuboid in shape and the follicular lumen was filled with PAS-positive colloid (Fig. 12).

Discussion

The present study, irrespective of the lack of a control specimen, evidently showed pathological changes in some visceral organs of a fresh dead body of an ancient murrelet : a marked necrosis in the proventricular epithelium, a sign of necrosis in some of the esophageal glands, and adrenocortical hyperplasia. These changes have been included in the symptoms of the oil-polluted birds reported thus far (Hartung and Hunt 1966 ; Guillon, 1967). Of the changes reported in the present paper, the necrotic change in the proventriculus may have been an important factor contributing to the death of the polluted seabirds. Apart from the direct physical effects of oiling on the plumage, the oil exerts toxic effects on various internal organs, being ingested during preening (Nelson-Smith, 1970). According to Hartung (1963), the experimentally oiled ducks preen approximately half the polluting oil from their feathers within a week, most of it on the first day of oiling. Furthermore, Hartung and Hunt (1966) reported that the oils fed to ducks caused lipid pneumonia, severe intestinal irritation, fatty changes in the liver, necrosis, and adrenal enlargement. Nervous abnormalities, suggesting inhibition of anti-cholinesterase activity, were probably due to organic phosphate additives in the samples of diesel and cutting oils. In the present case, the pollutant on the plumage had been ingested through preening and remained in the stomach, particularly in the proventriculus, which functions as the primary food reservoir. The epithelium of the proventriculus may be sensitive to the pollutant, because it is not provided with the tough coat, i.e., the cuticle layer, that is seen in the epithelium of the gizzard. On the other hand, the ingested pollutant merely passed through the esophagus and hence may have minimally affected its epithelium in comparison with its effects on the proventriculus. Severe derangement or irritation of the proventriculus caused by ingested petroleum may have subsequent and serious effects on the birds, e.g., suppression of food intake. Thus, it is assumed that the birds gradually become emaciated to the point of death in the cold sea during occasional stormy weather (Chiba, 1974). The low body weigth and almost no fat deposition under the skin and in the coelomic cavity may support this assumption.

A brief discussion should be extended to the adrenocortical hyperplasia observed in the present specimen. Hartung and Hunt (1966) reported great enlargement of the adrenal gland in many of experimental ducks that had been fed more than 1 ml/kg of diesel or cutting oil. This enlargement was obviously due to the hyperplasia of the cortical tissue, as the medullary one was affected only minimally. They also reported a similar picture of the adrenal gland from autopsied oil-killed wild ducks (Hartung and Hunt, 1966). Their data and the present findings are in accord and are interpreted as a general adaptation syndrome in response to stress (Selye, 1946). It is well established that in birds corticosterone plays an important role in carbohydrate, lipid, and electrolyte metabolism (Hodges, 1981). The adrenocortical hyperplasia in general association with the increased level of plasma corticosteroids may be involved in the heat production by the oiled birds (McEwan and Koelink, 1973), presumably by increasing the basic metabolism. This possibility should be analyzed experimentally in the future.

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